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(54) Title: CARNITINE RETENTION

(57) Abstract: A composition for influencing carnitine retention in biological tissue is disclosed. The composition comprises a carnitine substance and an agent to increase sodium potassium ATPase pump activity in the tissue, and/or to increase the activity of a carnitine transport protein, and/or to increase blood/plasma insulin concentration.

Carnitine Retention

This invention relates to carnitine retention in biological tissue. More particularly, but not exclusively, the invention relates to compositions and methods of increasing carnitine retention in the animal and/or human body.

It is known that carnitine is essential in muscle metabolism and function. In particular the muscle store of carnitine is important for energy production in muscle. If the store of carnitine declines, the function of the muscle can be impaired. Indeed, patients with muscle carnitine deficiency experience premature fatigue and weakness.

Previous studies (Harper *et al*, 1988, Segre *et al* 1988, Rebouche 1991), where oral doses of L-carnitine between 2 and 6 g were administered, demonstrate peak plasma concentrations ~3 h after ingestion and state a bioavailability of less than 20%. This poor absorptive status may be due to the fact that intestinal absorption of L-carnitine is normally near saturation (Taylor, 2001). Further studies (Rebouche *et al*, 1994, Brass *et al*, 1994) showed that if plasma carnitine concentrations exceed maximum renal reabsorption (60-100 µmol/L), the excess is excreted in the urine with a clearance approximating the glomerular filtration rate. From these features of carnitine's pharmacokinetics, and the fact that the normal plasma carnitine concentration of 40-50 µmol/L is sufficient to yield near maximal rates of skeletal muscle carnitine uptake (K_M 6.4 µM in isolated cells, Georges *et al*, 2000), it can be predicted that oral L-carnitine supplementation would have little, if any, impact on skeletal muscle carnitine content or metabolism in humans (Brass, 2000).

A study by Vukovich *et al* (1994) showed that L-carnitine supplementation (6 g every day for up to 2 weeks) resulted in no significant increase in resting skeletal muscle carnitine content and suggested that there was already an adequate amount of carnitine within the muscle to support fatty acid oxidation during exercise. However, Vukovich's study did not look at carnitine status in muscle. The results seen in a study by van Loon *et al*, 2001 which did look at

carnitine status in muscle do suggest, in contradiction to Vukovich, that there is not enough carnitine within the muscle to support fatty acid oxidation during exercise at workloads above 70% maximal oxygen consumption (VO_2 max). Other studies (Grieg *et al*, 1987, Oyono-Enguelle *et al*, 1988, Soop *et al*, 1988, Wyss *et al*, 1990, Decombaz *et al*, 1993), involved orally supplemented 3-5 g L-carnitine, in subjects with varying levels of fitness, over 5-28 days and measured the effects on various endpoints of exercise. Findings from these studies concluded that there was no effect of L-carnitine on VO_2 max, RQ, maximal exercise, fatty acid utilisation, glucose utilisation, lactate, perceived exertion, or heart rate. However, again these studies did not measure skeletal muscle carnitine content. If skeletal muscle carnitine content did not increase then clearly there would not be an affect on skeletal muscle metabolism and thus; an enhancement in the endpoints measured.

In contrast to these findings, Marconi *et al* (1985) did observe a slight but significant increase in VO_2 max in competitive walkers, after oral supplementation of 4 g L-carnitine every day for 2 weeks, which they concluded was most likely due to an increase in TCA flux as lipid metabolism did not change. Vecchiet *et al* (1990) also observed an increase in VO_2 max. However, only a single dose (2 g, orally) was supplemented an hour before exercise and, due to the features of carnitine's pharmacokinetics, it is highly unlikely the observed effects were a result of an increase in skeletal muscle carnitine, which was not measured.

According to one aspect of the present invention there is provided a composition for influencing carnitine retention in biological tissue, the composition comprising a carnitine substance and an agent to increase sodium-potassium ATPase pump activity in the tissue.

The invention further provides a composition for influencing carnitine transport into biological tissue, the composition comprising a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

According to a further aspect of the present invention there is provided a composition for increasing carnitine retention in the animal and/or human body, the composition comprising a carnitine substance and an agent to increase blood/plasma insulin concentration.

The invention also provides a method of influencing carnitine retention in biological tissue, in particular tissue of the animal and/or human body, the method comprising administering to the tissue a carnitine substance and an agent operable to increase sodium-potassium ATPase pump activity in the tissue.

The invention further provides a method of increasing carnitine retention in the animal and/or human body, the method comprising administering to the body a carnitine substance and an agent to increase blood/plasma insulin concentration.

The invention still further provides a method of influencing carnitine transport into biological tissue, the method comprising administering to the body a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

Preferably the method increases carnitine retention in the tissue by increasing the transportation of the carnitine substance, or a derivative thereof into tissue cells. Preferably transportation is increased by stimulation of a sodium dependent transport protein and substantially simultaneously increasing blood/plasma carnitine concentration.

Preferably the agent is operable to increase sodium dependent carnitine uptake into tissue cells, in particular skeletal muscle, liver and/or kidney cells.

The agent may be operable to increase insulin activity in the tissue, desirably by increasing the amount of insulin in the blood/plasma. The agent

may comprise carbohydrate or an active derivative thereof. Alternatively, or in addition, the agent may comprise amino acid and/or protein.

Preferably the method involves oral administration and desirably ingestion of the carnitine substance and agent, desirably but not necessarily simultaneously.

According to a still further aspect of the present invention there is provided a food supplement comprising a carnitine substance and an agent as described in any of the preceding paragraphs.

The invention further provides a composition for use in the manufacture of a medicament for influencing carnitine retention in biological tissue, the composition comprising a carnitine substance and an agent to increase sodium-potassium ATPase pump activity in the tissue.

The invention also provides a composition for use in the manufacture of medicament for influencing carnitine transport into biological tissue, the composition comprising a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

There is also provided a composition for use in the manufacture of a medicament to influence carnitine retention in the animal and/or human body, the composition comprising a carnitine substance and an agent to stimulate insulin release and activity in the body.

The invention also relates to the use of a carnitine substance and an agent as described in any of the preceding paragraphs for influencing carnitine retention in human and/or animal tissue.

Carnitine is also provided for use in a method substantially as described in any of the paragraphs above.

A kit is provided according to this invention comprising a carnitine substance and an agent substantially as described in any of the paragraphs above.

According to another aspect of the present invention, there is provided a carnitine substance for use in administration to the human and/or animal body with an agent as described in any of the paragraphs above.

Desirably, the carnitine substance comprises one or more of carnitine, a functional equivalent of carnitine, an active derivative of carnitine or carnitine analogue. A preferred embodiment may comprise one or more of L-carnitine, a functional equivalent of L-carnitine, an active derivative of L-carnitine or an analogue thereof.

Preferably the agent is a carbohydrate or a derivative of a carbohydrate. The carbohydrate is preferably a simple carbohydrate, which may be a simple sugar. Conveniently, the carbohydrate comprises glucose, but other sugars can be used, for example sucrose or fructose.

Desirably between 10 and 150 times the amount by weight of carbohydrate is administered to one unit of carnitine substance. Preferably between 10 and 95 times, and more preferably between 10 and 40 times, the amount by weight of carbohydrate is administered to one unit of carnitine substance. Desirably at least 0.25g of the carnitine substance is administered, preferably with at least 2.5g of the agent. Conveniently substantially 0.25g of the carnitine substance is administered desirably with between substantially 2.5g and 37.5g of the agent, preferably between substantially 2.5g and 23.75g, and more preferably between substantially 2.5g and 10g of the agent. Conveniently, substantially 3g of the carnitine substance is administered, desirably with between substantially 30g to 450g of the agent, preferably between substantially 230g and 285g, and more preferably between 30g and 120g of the agent. Conveniently substantially 0.25g to 3 g of the carnitine substance is administered, desirably with a total of between substantially 2.5g to 450 g of the

agent, preferably between substantially 2.5g and 285 g, and more preferably between substantially 2.5g and 120 g of the agent. The agent may be administered to achieve substantially simultaneous elevation of insulin and carnitine concentrations in the blood/plasma.

The composition may be provided in a solution which may be an aqueous solution.

Embodiments of the invention will now be described by way of example only with reference to the accompanying drawings, in which:-

Fig. 1 shows serum insulin concentrations for Example I following carnitine ingestion with Control (○) and CHO (●);

Fig. 2 shows urinary total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) excretion in mg for Example I over 24 hours following carnitine ingestion with Control (□) and CHO (■);

Fig. 3 shows plasma TC concentration for Example I measured over 7 hours following carnitine ingestion with Control (□) and CHO (■). The arrows A, B, C, D indicate time of ingestion of drink;

Fig. 4 shows the area under the plasma-time curves (AUC) for Example I for total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) measured over 7 hours following carnitine ingestion with Control (□) and CHO (■).

Fig. 5 is a block diagrammatic representation of the study protocol of Example II;

Fig. 6 shows serum insulin concentrations for Example II during 6 hour intravenous insulin infusions of 5 (□), 30 (■), 55 (○) and 105 (●) mU m^{-2} . min^{-1}, combined with a 5 hour intravenous 60 mM L-carnitine infusion;

Fig. 7 shows plasma total carnitine concentrations during 6 hour intravenous insulin infusions of 5 (□), 30 (■), 55 (○) and 105 (●) mU .m^{-2}.min^{-1};

Fig. 8 shows the plasma carnitine data for Example II for the 5 (○) and 105 (●) mU.m^{-2}.min^{-1} doses;

Fig. 9 shows plasma a total carnitine concentration of Example III during 6 hour intravenous infusion of 5 (o) and 105 (•) $mU.m^{-2}.min^{-1}$ combined with a 5 hour intravenous 60 mM L-carnitine infusion; and

Fig. 10 shows muscle acy, acetyl and free carnitine concentrations for Example III pre and post 6 hour intravenous insulin infusions of 5 and 105 $mU.m^{-2}.min^{-1}$ combined with a 5 hour intravenous 60mM L-carnitine infusion.

Referring to the figures, the invention provides a composition, methodology and uses of a composition to influence carnitine retention in tissue such as muscle, liver and kidney tissue in the animal and/or human body which comprise a carnitine substance and an agent to increase blood/plasma insulin concentration with a view to increasing sodium-potassium ATPase pump activity in tissue, and thereby sodium dependent carnitine transport.

The carnitine substance comprises one or more of L-carnitine, a functional equivalent of L-carnitine, an active derivative of L-carnitine or an analogue thereof.

The agent can be anything which acts to increase insulin concentration, including amino acids and protein. However in this embodiment the agent is a carbohydrate such as a sugar, for example glucose which acts to stimulate insulin production in the body.

Example I

Eight, healthy, moderately trained, non-vegetarian men (age 22.3 ± 0.7 yr, body mass 79.7 ± 2.5 kg, and body mass index 24.3 ± 0.9 kg/m^2) were used in the study of this Example.

The study protocol utilised a blind crossover design where subjects acted as their own controls. Following an overnight fast, subjects reported to the laboratory on two occasions, separated by a 2 week "wash out" period to ensure similar basal muscle carnitine concentrations among experimental treatments.

On arrival, subjects voided their bladder and were asked to rest in a supine position on a bed while a cannula was inserted retrogradely into a superficial vein on the dorsal surface of the non-dominant hand. This hand was kept in a hand-warming unit (air temperature 55°C) to arterialise the venous drainage of the hand and a saline drip was attached to keep the cannula patent.

After a basal blood sample was taken, subjects consumed 3.01 g (3 x 1.5 g L-carnitine L-tartare effervescent tablets) L-carnitine (Lonza Group, Basel, Switzerland) dissolved in 200 ml of water. After 1 hour and then 3 more times every 1.5 hours (h), subjects consumed a 500 ml drink over a 5 min period in a randomised order containing either sugar free orange drink (Control) or 94 g of simple sugars (CHO) (Original Lucozade, GlaxoSmithKline, Brentford, UK).

Subjects abstained from the consumption of meat, dairy produce, alcohol, and strenuous exercise 24 hours before each visit and for a 24 hour period after the consumption of the carnitine solution. It was essential that subjects had a minimal intake of carnitine in their diet during this period; therefore, food was supplied to the subjects as a ready-made meal, free from carnitine.

During each experimental visit, 5 ml of arterialised venous (a-v) blood were obtained every 20 min for 7 h after which subjects left the laboratory, returning for a final 24 hour blood sample. Two ml of this blood were collected into lithium heparin containers and, after centrifugation (14,000 rpm for 2 min), the plasma was removed and immediately frozen in liquid nitrogen. These samples were then stored at -80°C and analysed for free and total carnitine concentrations at a later date. The remaining blood was allowed to clot, and, after centrifugation (3,000 rpm for 10 min), the serum was stored frozen at -20°C. Insulin concentration was measured in these samples at a later date with a radioammunoassay kit (Coat-a-Count Insulin, DPC, Ca, USA).

Urine was collected in 5 litre bottles, containing 5 ml of 10% thymol/isopropanol preservative, for 24 h following the consumption of the carnitine drink and returned to the laboratory the following morning where a final

blood sample was taken. The 24 h volume was recorded and 5 ml aliquots were removed and stored at -20°C to be analysed for free and total carnitine concentrations at a later date.

The method used for the determination of carnitine is based on the carnitine acetyltransferase (CAT) catalysed reaction:

L-carnitine + [^{14}C]acetyl-CoA ↔ [^{14}C]acetyl-L-carnitine + CoASH and measures the concentration of [^{14}C]acetyl-L-carnitine. The reaction is reversible, but the removal of CoASH via complex with N-ethylmalemide (NEM) ensures the reaction is driven quantitatively to the right and that all the L-carnitine is labelled. To separate labelled acetyl-L-carnitine from any remaining [^{14}C]acetyl-CoA Cederblad & Lindstedt (1972) introduced the use of anion-exchange resin. The negatively charged acetyl-CoA remains in the resin whereas the positive acetyl-L-carnitine is excluded for collection.

L-carnitine for use in the standards was purchased from Sigma Chemical Co., St. Louis, Mo. U.S.A., as was the unlabelled acetyl-coenzyme A (sodium salt, purity 90-95%), N-ethylmalemide, and the Dowex 1X 8 (200-400 mesh, Cl^- form). [^{14}C]acetyl-coenzyme A was obtained from Amersham, Buckinghamshire, UK (specific radioactivity 10 μCi). Carnitine acetyltransferase (5 mg/ml) was obtained from Roche Molecular Biochemicals, East Sussex, U.K. and scintillation liquid (Scintillator Plus) was purchased from Packard Biosciences, Groninger, The Netherlands.

All samples were analysed in duplicate. For plasma samples, 50 μl plasma were pipetted, by positive displacement, into a 3 ml glass test tube. After the addition of 1.2 ml chloroform/methanol ($CHCl_3$:CH_3OH, 3:2) the sample was vortexed, and then centrifuged at 4,500 rpm for 10 min. The supernatant was poured off to another glass tube while the pellet, after being broken up with a plastic rod, was ashed with a further 0.6 ml $CHCl_3$:CH_3OH, vortexed and centrifuged again (4,500 rpm, 10 min). This second supernatant was pooled with

the first and the sample was dried by the evaporation of the $CHCl_3$:CH_3OH under N_2.

For total carnitine, all of the acyl-carnitine bonds were hydrolysed by the addition of 100 µl 0.1 M KOH to the test tube. The sample was then placed in a water bath at 50°C for 2 h. After incubation 20 µl of 0.5 M HCl were added to neutralised the sample.

For free carnitine, 120 µl H_2O (Millipor) were added to make the free and total solutions of equal volume.

For urine samples 10 µl urine were pipetted, by positive displacement, into a 3 ml glass test tube and diluted with 40 µl urine were pipetted, by positive displacement, into a 3 ml glass test tube and diluted with 40 µl H_2O (Millipor). The sample then underwent the same procedure as the plasma sample.

For the preparation of standards, 15, 30, 45, 60, 75, and 90 µl of 40 µmol/l L-carnitine standard solution were pipetted into 3.5 ml test tubes and made up to 120 µl volume by adding H_2O (Millipor). This produced 7 standards with L-carnitine concentrations of 0, 600, 1200, 1800, 2400, 3000, and 3600 pmol/l.

Radioenzymatic analysis of carnitine was carried out by adding twenty-five µl phosphate buffer (1 M, pH 6.5), 25 µl acetyl-CoA (300 µM), 10 µl NEM (40 mM), and 25 µl (^{14}C]acetyl-CoA (4 µM) to each tube. Carnitine acetyltransferase (CAT) diluted 1:10 was then defrosted and 20 µl were added to each sample at 20 s intervals. After 30 min incubation at room temperature the contents of each tube were transferred to a column of Dowex 1X 8 contained in a Pasteur pipette at 20 s intervals using an automatic pipette (Microlab 1000, Hamilton, Bonaduz, Switzerland). The pipette aspirated each 240 µl sample with a 10 µl air gap, mixed with 250 µl water (Millipor) and then voided the solution into the top of column. The tube was then washed with 250 µl water (Millipor), which were then aspirated, mixed with 250 µl water (Millipor) and voided into the same column.

The effluent was collected into 20 ml vials and mixed with 10 ml scintillation fluid. β-radioactivity of each of the vials was counted for 3 min.

A two-way ANOVA (time and treatment effects, SPSS version 10, USA) was performed to detect differences in plasma carnitine and serum insulin. A Student's paired *t*-test was used to locate differences in 24 h urinary carnitine content and area under plasma time curved between treatments. The total area under the plasma carnitine-time curve was calculated using KaleidaGraph (version 3.51, Synergy Software, USA). Statistical significance was declared at $P < 0.05$, and all the values are means ± SE.

The results will now be discussed with particular reference to the drawings.

Fig. 1 shows a plot of serum insulin concentrations following carnitine ingestion with Control CON (○) and carbohydrate CHO (●). Insulin concentration was significantly higher ($P<0.01$) following ingestion of four 500 ml drinks in the carbohydrate group (94 g simple sugars indicated by arrows A, B, C, D at t = 60, 150, 240 and 330) than in the control group (sugar free indicated by arrows t = 60, 150, 240 and 330). Values are ± SE expressed in mU/l (n = 8).

Fig. 2 shows a graph of urinary total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) excretion in mg over a period of 24 hours following an oral dose of 3.01 g of L-carnitine ingestion with control (CON) and carbohydrate (CHO). Mean urinary TC, FC and AC secretion was reduced when subjects consumed CHO compared to Control, and * indicates that excretion was significantly lower in the case of TC and AC ($P<0.05$). Values are means ± SE expressed in mg/24 hr (n = 8).

Fig. 3 shows a plot of plasma total carnitine concentration measured over 7 hours following an oral dose of 3.01 g L-carnitine with Control (□) and carbohydrate (■).

The arrows A, B, C and D indicate time of ingestion of drink. No significant differences ($P<0.05$) were seen between the two groups (Control and CHO), either at basal or at any point following ingestion. Values are means ± SE expressed in µmol/l (n = 8).

Fig. 4 shows a plot of the area under the plasma-time curves (AUC) for total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) measured over a 7 hour period following an oral dose of 3.01 g L-carnitine with Control (CON) and carbohydrate (CHO). No significant differences were seen in TC and FC AUC's when comparing Control and carbohydrate, but AC was significantly lower ($P<0.05$) following CHO, resulting in a significant decrease in plasma carnitine concentration with CHO. Values are means ± SE expressed in mmol/l/min (n = 8).

The results show that L-carnitine supplementation together with CHO results in a smaller loss of urinary carnitine than that seen with Control. Total (TC), free (FC) and acyl (AC) carnitine were all excreted less with CHO, than in Control.

From the results it can be seen that insulin, released as a result of ingesting carbohydrate (CHO), stimulates L-carnitine retention. Insulin increases carnitine retention most probably by increasing sodium-potassium ATPase pump activity and, thus, sodium dependent transport of carnitine into cells (particularly skeletal and cardiac muscle). Insulin may also enable more FC to be available to tissues by 1) inhibiting acylation of supplemented L-carnitine and/or 2) by stimulating carnitine retention by reabsorption by the kidney.

Example II

Seven, healthy, moderately trained, non-vegetarian men (age 20.3 ± 0.4 yr, body mass 76.4 ± 3.1 kg, and body mass index 23.7 ± 1.0 kg / m^2) participated in the study of this Example.

The study protocol was as follows.

Subjects reported to the laboratory, following an overnight fast on four occasions, each separated by a ≥ 2 wk "carnitine wash out" period, having abstained from meat, alcohol and strenuous exercise for the previous 24 h and having voided their bladder immediately prior to entering the laboratory. On arrival, subjects were asked to rest in a semi-supine position on a bed while a cannula was inserted retrogradely in to a superficial vein on the dorsal surface of the non-dominant hand. This hand was kept in a hand-warming unit (air temperature 55°C) to arterialise the venous drainage of the hand and a saline drip was attached to keep the cannula patent. A second cannula was placed in an antecubital vein in the non-dominant forearm for the infusion of insulin and glucose and a third cannula was inserted into an antecubital vein in the opposite arm for infusion of L-carnitine.

On each experimental visit a 360 min euglycemic insulin (human Actrapid) clamp was performed, whilst maintaining a blood glucose concentration of 4.4 ± 0.01 mmol / 1 via infusion of a 20% glucose solution. The insulin clamp began at t = 0 (Fig.5) and varied between visits being either 5, 30, 55, or 105 mU. $M^{-2}.min^{-1}$ in order to obtain a fasting, fed, physiologically high, or close to supraphysiological serum insulin concentration, respectively. Following a 60 min equilibration period, an intravenous infusion of 60 mM L-carnitine (Lonza Group, Basel, Switzerland) began in conjunction with the insulin clamp, which lasted for the remainder of the protocol (Fig. 5). Specifically, a bolus dose of 15mg – kg^{-1} L-L-carnitine was administered intravenously over a 10 min period in order to achieve a plasma concentration of - 500 µmol / 1. This was followed by a constant infusion at 10mg . kg^{-1} . h^{-1} for the next 290 min to maintain a supraphysiological steady steady state plasma carnitine concentration. At t = 360 both insulin and L-carnitine infusions were stopped and subjects were free to leave the laboratory once their blood glucose levels had stabilised.

With reference to Fig. 5 it should be noted that euglycaemia was maintained throughout insulin infusion by means of simultaneous infusion of a

20% glucose solution. During each of the four experimental visits, 1 ml of arterialized venous (a-v) blood was obtained every 5min to determine blood glucose concentration on-line (YSI 2300 STATplus, YSI, Yellow Springs, OH). In addition, 5 ml of a-v blood were obtained every half hour (and at 80 min) for 6 h. Two ml of this blood were collected into lithium heparin containers and, after centrifugation (14,000 rpm for 2 min), the plasma was removed and immediately frozen in liquid nitrogen. These sampled were then stored at -80°C and analysed for free and total carnitine concentrations at a later date using a radio enzymatic assay. The remaining blood was allowed to clot, and after centrifugation (3,000 rpm for 10 min), the serum was stored frozen at 20°C. Insulin was measured on these sample at a later date using a radioammnassy kit (Coat-a-Count Insulin, DPC, Ca, USA).

A two-way ANOVA (time and treatment effects, GraphPad Prism version 3, GraphPad Software, Inc., USA) was performed to detect differences in plasma carnitine and serum insulin concentrations. If significance was achieved, a repeated measure ANOVA (GraphPad Prism version 3, GraphPad Software, Inc., USA) was used to locate differences between treatments at each time point. Statistical significance was declared at $P < 0.05$, and all values presented represent mean ± standard error (SE).

The results will now be discussed with reference to Figs 5 to 7 of the drawings.

Following the 60 min equilibration period, steady-state serum, insulin concentration for each of the four insulin infusion protocols (5, 30, 55 and 105mU . m^{-2} . min^{-1}) was 10.3 ± 0.3, 47.8 ± 1.3, 85.6 ± 2, 198.6 ± 4.8 mU / 1, respectively (Fig 6). Steady-state serum insulin concentration was markedly different between each of the treatment groups (statistical differences not shown in Fig. 6 for the sake of clarity).

Insulin concentrations during 6 h intravenous insulin infusions of 5 (□), 30 (■), 55 (○) , and 105 (●) mU . m^{-2} . min^{-1} combined with a 5 h intravenous 60 mM

L-carnitine infusion. Values are means ± SE expressed in mU / 1. Statistical differences not shown in Fig. 6 for the sake of clarity.

Plasma total carnitine (TC) concentration before and throughout the 300 min of 60 mM L-carnitine infusion during each fo the four euglycaemic insulin clamps (5, 30, 55, and 105 mU . m^{-2} . min^{-1}) is shown in Fig. 7. The basal plasma TC concentration was similar across experimental groups, (i.e. 47.7 ± 0.6 µmol / 1). The 10 min bolus L-carnitine infusion (15 mg – kg^{-1}) markedly increased plasma TC concentration to 545.5 ± 16.4, 546.8 ± 20.7, 559.4 ± 41.2, and 509.7 ± 17.4 µmol / 1 during each of the insulin clamps (5, 30, 55 and 105 mU . m^{-2} . min^{-1} , respectively). Plasma TC concentrations fell slightly in each experimental group when the L-carnitine infusion rate was reduced to 10 mg . kg^{-1} . h^{-1} (see t = 120, Fig. 7), but was thereafter maintained at steady state concentrations well above basal (Fig. 7). Differences in plasma TC concentration between experimental groups became evident during the final 2 h of carnitine infusion. The plasma TC concentration during the 105 mU . m^{-2} . min^{-1} insulin infusion was significantly lower than the 5 mU . m^{-2} . min^{-1} insulin infusion at t = 240 ($p< 0.05$), t = 300 ($p< 0.05$), and t = 360 ($p< 0.01$; Fig. 3.) Similarly, the plasma TC concentration during 105 mU . m^{-2} . min^{-1} insulin infusion was also significantly lower than during the 30 mU . m^{-2} . min^{-1} clamp at t =360 ($p< 0.05$).

Fig. 7 shows the plasma total carnitine concentrations during 6 h intravenous insulin infusions of 5(□), 30 (■), 55 (○), and 105 (●) mU . m^{-2} . min^{-1} combined with a 5 h intravenous 60 mM L-carnitine infusion. Values are means ± SE expressed in µmol/l.

To highlight the effect of insulin on plasma carnitine concentration, Fig. 8 shows the plasma carnitine data for only the 5 and 105 mU . m^{-2} . min^{-1} doses. As can be seen, plasma carnitine concentration was siginificantly lower during the final two hours of infusion at the highest dose.

This study maintains a supra-physiological steady state plasma carnitine concentration for 5 h and also combines this with varying steady state serum insulin concentrations.

During 5 h of L-carnitine infusion, plasma total carnitine concentration in a 105 mU . m^{-2} . min^{-1} euglycaemic insulin clamp was lower than during a 5 and 30 mU . m^{-2} . min^{-1} insulin clamp. This clearly demonstrates that L-carnitine clearance from plasma,either into the urine or periphery, is increased in the presence of high serum insulin levels.

Thus, it would appear that the high serum insulin concentration increased sodium dependent L-carnitine transport into skeletal muscle via activation of the Na^+-K^+ATPase pump, resulting in the observed fall in plasma total carnitine.

Example III

Eight, healthy, moderately trained, non-vegetarian men participated in the present study.

The study protocol was as follows. Subjects reported to the laboratory in the morning after an overnight fast and underwent exactly the same experimental procedures as described in the previous Example II study protocol. However, on this occasion two, as opposed to four, euglycaemic insulin clamps (5 and 105 mU . m^{-2} . min^{-1}) were performed in a randomised order, and each was seprated by 2 weeks. Each clamp was maintained for 6 hours and a muscle biopsy sample was obtained from the quadriceps muscle group in the basal state (prior) to infusion of carnitine and glucose and insulin) and after 6 hrs of infusion. Analytical and statistical procedures were as described above, with the exception of muscle acyl, acetyl and free carnitine carnitine concentrations which were analysed according to the method of Cederblad et al. Statistical differences in muscle carnitine status was determined using Student's Paired T-test.

The results will now be discussed with reference to Fig. 9 of the drawings which shows plasma carnitine concentration during the 5 and 105 mU . m^{-2} . min^{-1} insulin clamps. In keeping with the response observed in the previous experiment (Fig. 8), plasma carnitine concentration was significantly lower at the highest insulin infusion does.

Fig. 9 shows the plasma total carnitine concentrations during 6 h intravenous insulin infusions of 5 (○) and 105 (●) 105 mU . m^{-2} . min^{-1} combined with a 5 h intravenous 60 mM L-carnitine infusion. Values are means ± SE expressed in µmol / 1. Statistical differences between treatments: ** $p<0.01$, *** $P<0.001$.

Fig. 10 shows muscle acyl, acetyl and free carnitine concentrations (sum equals muscle total carnitine concentration) pre and post 6 h intravenous insulin infusions of 5 and 105 (●) mU . m^{-2} . min^{-1} combined with a 5 h intravenous 60 mM L-carnitine infusion. Values are means ± SE expressed in µmol / 1. Statistical differences between treatments: * $p<0.05$.

The lowest insulin infusion rate (equivalent to fasting insulin concentration) had no effect on muscle carnitine accumulation. However, the highest infusion rate resulted in a significant increase in muscle total carnitine concentration.

These findings conclusively demonstrate that:

(i) Carnitine per se does not readily enter the muscle compartment (even when plasma carnitine concentration is dramatically elevated). This observation is in keeping with the notion that carnitine supplementation per se does not elevate the muscle carnitine pool.

(ii) Insulin promotes muscle carnitine accumulation in the presence of elevated plasma carnitine concentrations. This is the first demonstration that insulin can have such an effect. We believe this response is achieved via the stimulatory effect of insulin on sodium dependent muscle carnitine transport. The present invention therefore has useful application in increasing carnitine retention in muscle tissue and thereby reducing the metabolic effects of depleted free carnitine in muscle during exercise, and including the effect on muscle fatigue and muscular performance.

Various modifications may be made without departing from the scope of the present invention. For example other agents may be used which stimulate carnitine retention primarily by way of increasing carnitine transport into tissue, such as insulin or active derivatives thereof. Other agents may include, either as an alternative or as an addition, amino acid(s) and protein(s). Active derivatives, variants or analogues of carnitine may be used. The composition may be administered in any convenient form such as tablet, powder, pellet or the like and otherwise than by ingestion, such as injection.

Between 10 and 150 times the amount by weight of agent such as carbohydrate may be administered to one unit of carnitine substance.

The invention can be used to increase carnitine retention in animal as well as human bodies, and in whole bodies, tissues or cells derived therefrom.

The invention also provides a kit comprising a carnitine substance and an agent such as a carbohydrate, as described above.

Whilst endeavouring in the foregoing specification to draw attention to those features of the invention believed to be of particular importance it should be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether or not particular emphasis has been placed thereon.

CLAIMS

1. A composition for influencing carnitine retention in biological tissue, the composition comprising carnitine substance and an agent to increase sodium potassium ATPase pump activity in the tissue.

2. A composition for influencing carnitine transport into biological tissue, the composition comprising a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

3. A composition for increasing carnitine retention in the animal and/or human body, the composition comprising a carnitine substance and an agent to increase blood/plasma insulin concentration.

4. A composition for use in the manufacture of a medicament for influencing carnitine retention in biological tissue, the composition comprising a carnitine substance and an agent to increase sodium-potassium ATPase pump activity in the tissue..

5. A composition for use in the manufacture of medicament for influencing carnitine transport into biological tissue, the compositon comprising a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

6. A composition for use in the manufacture of a medicament to influence carnitine retention in the animal and/or human body, the composition comprising a carnitine substance and an agent to stimulate insulin release in the body.

7. A composition according to any preceding claim wherein the agent is operable to increase sodium dependent carnitine uptake into tissue cells, in particular skeletal muscle, liver and/or kidney cells.

8. A composition according to any preceding claim wherein the agent is operable to increase insulin activity in the tissue.

9. A composition according to claim 8 wherein the agent is operable to increase insulin activity in the tissue by increasing the amount of insulin in the blood/plasma.

10. A composition according to any preceding claim wherein the agent comprises a carbohydrate, and/or an active derivative thereof, and/or an amino acid and/or a protein.

11. A composition according to claim 10 wherein the agent is a carbohydrate and/or a derivative of a carbohydrate.

12. A composition according to claim 10 or 11 wherein the carbohydrate is a simple carbohydrate, and/or the derivative of the carbohydrate is a derivative of a simple carbohydrate.

13. A composition according to claim 11 wherein the carbohydrate is a simple sugar, and/or the derivative of the carbohydrate is a derivative of a simple sugar.

14. A composition according to claim 12 or 13 wherein the carbohydrate comprises glucose, sucrose, and/or fructose, and/or the derivative of the carbohydrate is a derivative of glucose, sucrose and/or fructose.

15. A composition according to any preceding claim wherein the amount by weight of the agent is between 10 and 150 times the amount by weight of the carnitine substance.

16. A composition according to any preceding claim wherein the amount by weight of the agent is between 10 and 95 times the amount by weight of the carnitine substance.

17. A composition according to any preceding claim wherein the amount by weight of the agent is between 10 and 40 times the amount by weight of the carnitine substance.

18. A composition according to any preceding claim comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 450g of the agent.

19. A composition according to any preceding claim comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 285g of the agent.

20. A composition according to any preceding claim comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 120g of the agent.

21. A composition according to any preceding claim in the form of a solution.

22. A composition according to any preceding claim in the form of an aqueous solution.

23. A food supplement comprising a carnitine substance and an agent to increase the sodium-potassium A T Pase pump activity in the tissue.

24. A food supplement comprising a carnitine substance to increase blood/plasma carnitine concentration, and an agent to increase the activity of a carnitine transport protein.

25. A food supplement comprising a carnitine substance and an agent to increase blood/plasma insulin concentration.

26. A food supplement according to claims 23, 24 or 25 wherein the agent is operable to increase sodium dependent carnitine uptake into tissue cells, in particular skeletal muscle, liver and/or kidney cells.

27. A food supplement according to any of claims 23 to 26 wherein the agent is operable to increase insulin activity in the tissue.

28. A food supplement according to claim 27 wherein the agent is operable to increase the insulin activity in the tissue by increasing the amount of insulin in the blood/plasma.

29. A food supplement according to any of claims 23 to 28 wherein the agent comprise a carbohydrate, and/or an active derivative thereof and/or an amino acid and/or a protein.

30. A food supplement according to claim 29 wherein the agent is a carbohydrate and/or a derivative of a carbohydrate.

31. A food supplement according to claim 29 or 30 wherein the carbohydrate is a simple carbohydrate and/or the derivative of the carbohydrate is a derivative of a simple carbohydrate.

32. A food supplement according to claim 31 wherein the carbohydrate is a simple sugar, and/or the derivative of the carbohydrate is a derivative of a simple sugar.

33. A food supplement according to claim 30, 31 or 32 wherein the carbohydrate comprises glucose, sucrose and/or fructose, and/or the derivative of the carbohydrate comprises a derivative of glucose, sucrose and/or fructose.

34. A food supplement according to any of claims 23 to 33 wherein the amount by weight of the agent is between 10 and 150 times the amount by weight of the carnitine substance.

35. A food supplement according to claims 23 to 34 wherein the amount by weight of the agent is between 10 and 95 times the amount by weight of the carnitine substance.

36. A food supplement according to claims 23 to 35 wherein the amount by weight of the agent is between 10 and 40 times the amount by weight of the carnitine substance.

37. A food supplement according to claims 23 to 36 comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 450g of the agent.

38. A food supplement according to any of claims 23 to 37 comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 285g of the agent.

39. A food supplement according to any of claims 23 to 38 comprising substantially 0.25g to 3g carnitine substance and between 2.5g and 120g of the agent.

40. A food supplement according to claims 23 to 39 in the form of a solution.

41. A food supplement according to any of claims 23 to 40 in the form of an aqueous solution.

42. A method of influencing carnitine retention in biological tissue, in particular tissue of the animal and/or human body, the method comprising administering to the tissue a carnitine substance and an agent operable to increase sodium-potassium ATPase pump activity in the tissue.

43. A method of increasing carnitine retention in the animal and/or human body, the method comprising administering to the body a carnitine substance and an agent to increase blood/plasma insulin concentration.

44. A method of influencing carnitine transport into biological tissue, the method comprising administering to the body a carnitine substance to increase blood/plasma carnitine concentration and an agent to increase the activity of a carnitine transport protein.

45. A method according to any of claims 42 to 44 wherein the method increase carnitine retention in the tissue by increasing the transportation of the carnitine substance, or a derivative thereof into tissue cells.

46. A method according to claim 45 wherein transportation is increased by stimulation of a sodium dependent transport protein and substantially simultaneously increasing blood/plasma carnitine concentration.

47. A method according to any of claims 42 to 46 wherein the agent is operable to increase sodium dependent carnitine uptake into tissue cells, in particular skeletal muscle, liver and/or kidney cells.

48. A method according to any of claims 42 to 47 wherein the agent is operable to increase insulin activity in the tissue.

49. A method according to claim 48 wherein the agent is operable to increase insulin activity in the tissue by increasing the amount of insulin in the blood/plasma.

50. A method according to any of claims 42 to 49 wherein the agent comprises a carbohydrate, and/or an active derivative thereof, and/or an amino acid and/or a protein.

51. A method according to claim 50 wherein the agent is a carbohydrate and/or a derivative of a carbohydrate.

52. A method according to claim 50 or 51 wherein the carbohydrate is a simple carbohydrate, and/or the derivative of the carbohydrate is a derivative of a simple carbohydrate.

53. A method according to claim 52 wherein the carbohydrate is a simple sugar, and/or the derivative of the carbohydrate is a derivative of a simple sugar.

54. A method according to claim 52 or 53 wherein the carbohydrate comprises glucose, sucrose and/or fructose, and/or the derivative of the carbohydrate is a derivative of glucose, sucrose and/or fructose.

55. A method according to any of claims 42 to 54 wherein the method involves oral administration and ingestion of the carnitine substance and agent.

56. A method according to claim 55 wherein the oral administration and ingestion of the carnitine substance and the agent occurs simultaneously.

57. A method according to any of claims 42 to 56 wherein the amount by weight of the agent is between 10 and 150 times the amount by weight of the carnitine substance.

58. A method according to any of claims 42 to 57 wherein the amount by weight of the agent is between 10 and 95 times the amount by weight of the carnitine substance.

59. A method according to any of claims 42 to 58 wherein the amount by weight of the agent is between 10 and 40 times the amount by weight of the carnitine substance.

60. A method according to any of claims 42 to 59 wherein substantially 0.25g to 3g of the carnitine substance and between 2.5g and 450g of the agent are administered.

61. A method according to any of claim 42 to 60 when substantially 0.25g to 3g of the carnitine substance and between 2.5g and 285g of the agent are administered.

62. A method according to any of claims 42 to 61 wherein substantially 0.25g to 3g of the carnitine substance and between 2.5g and 120g of the agent are administered.

63. A composition substantially as herein described with reference to Example I, II or III.

64. A food supplement substantially as herein described with reference to Examples I, II or III.

65. A method substantially as here described with reference to Examples I, II or III.

66. Any novel subject matter or combination including novel subject matter disclosed herein, whether or not within the scope of or relating to the same invention as any of the preceding claims.

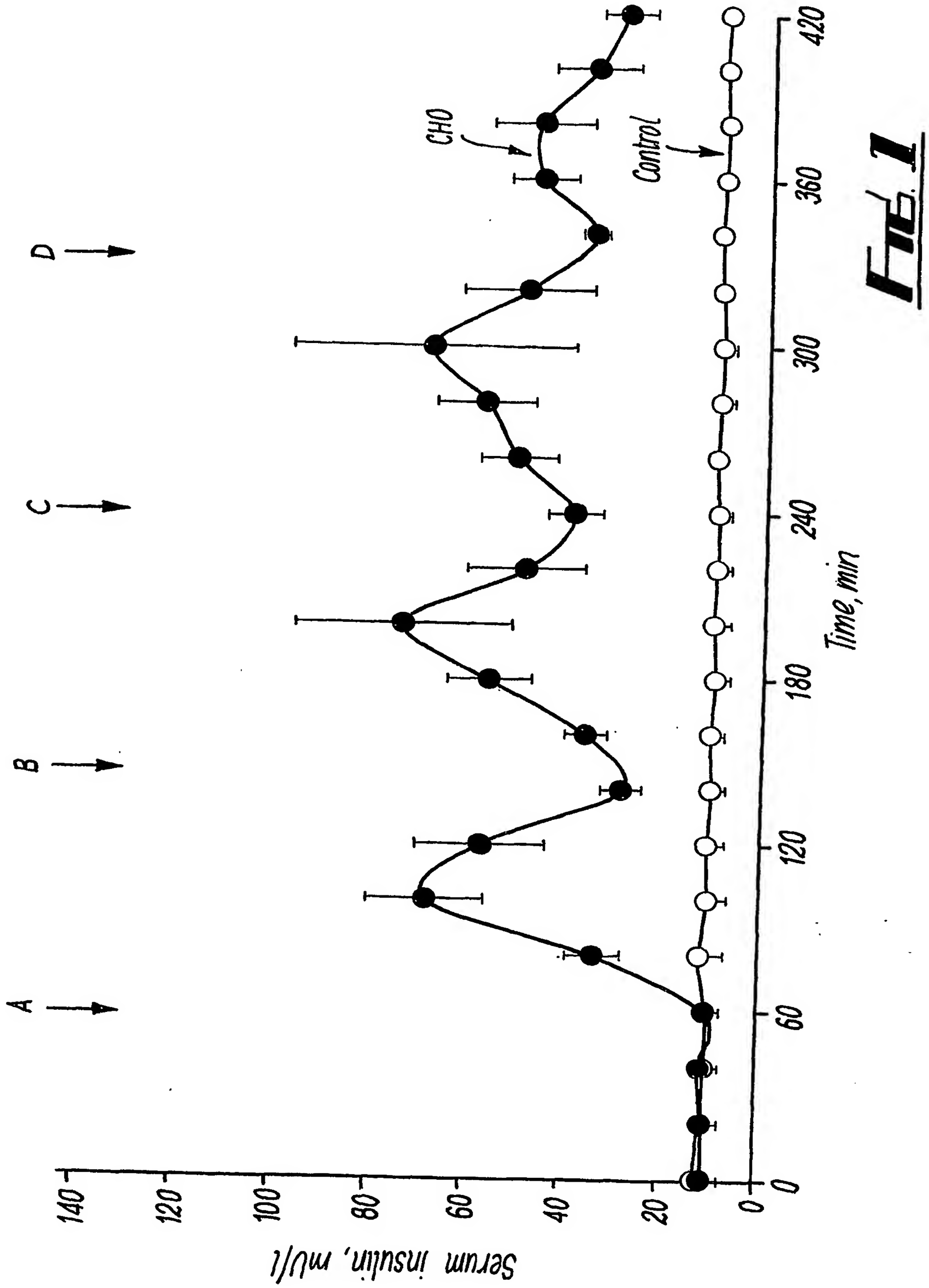

A
B
C
D
CHO
Control
Serum insulin, mU/l
Time, min
0
60
120
180
240
300
360
420
0
20
40
60
80
100
120
140

FIG. 1

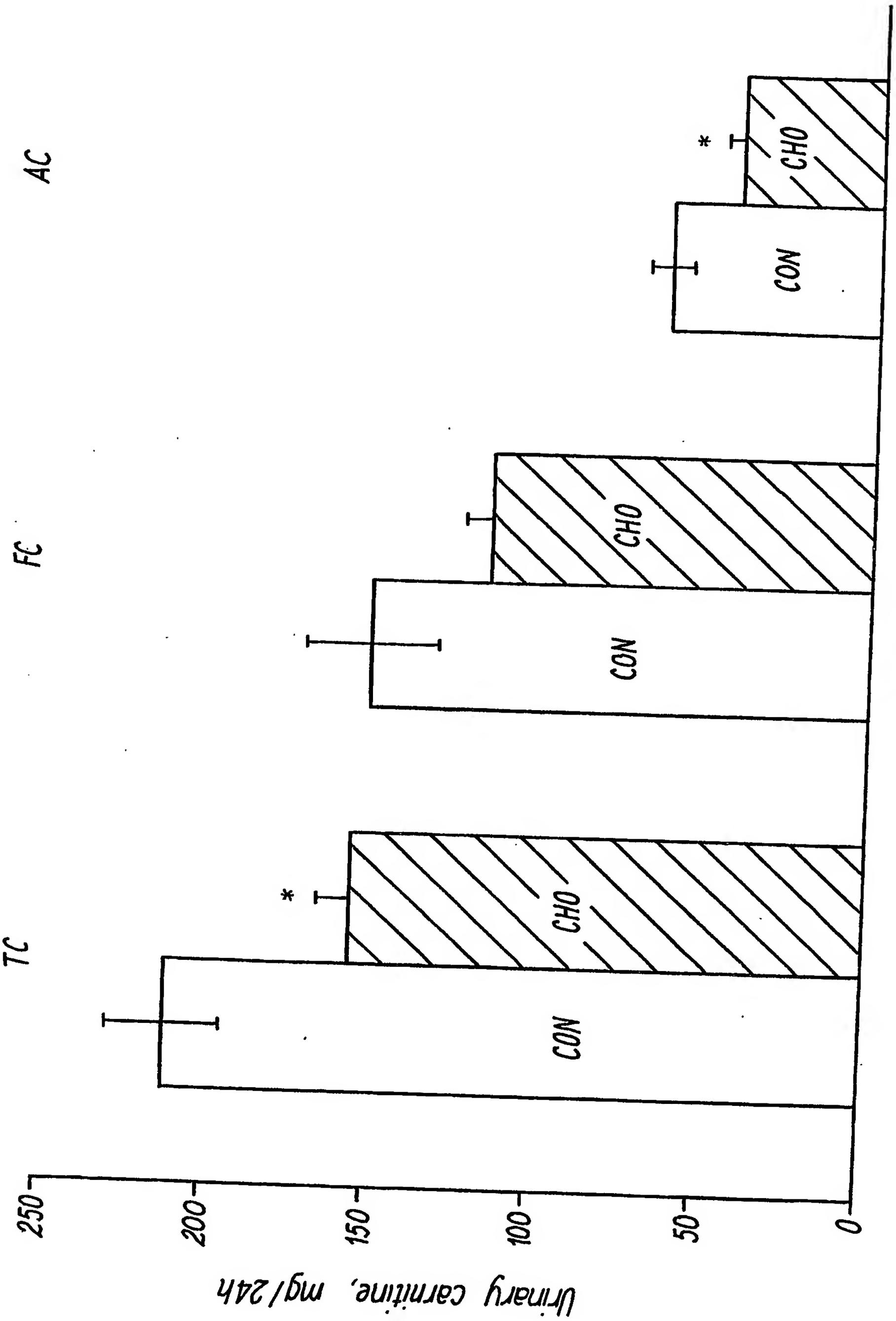


FIG 2

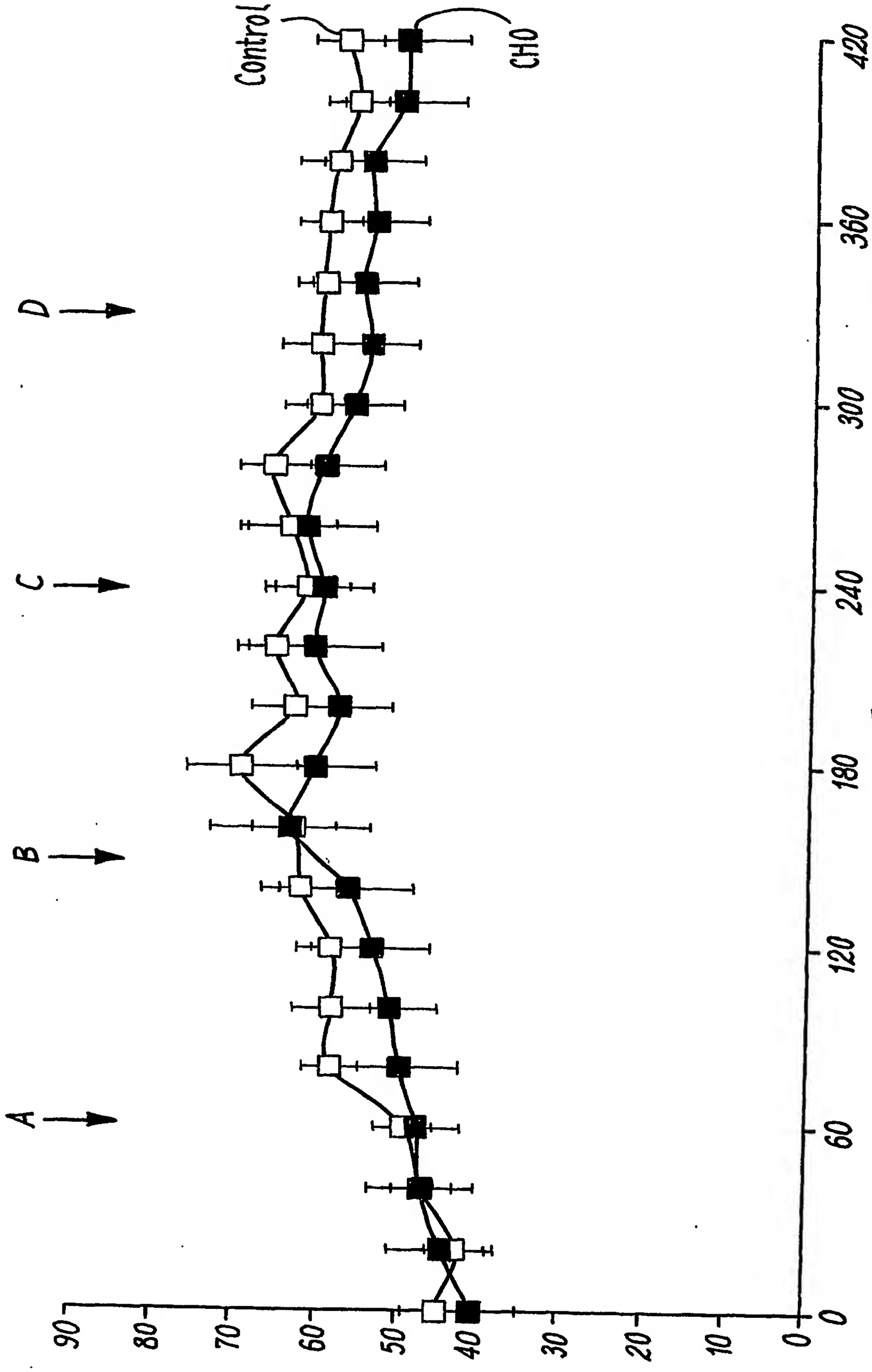

Control
CHO
A
B
C
D
Plasma total carnitine, umol/L
0
10
20
30
40
50
60
70
80
90
0
60
120
180
240
300
360
420
Time, min

FIG. 3

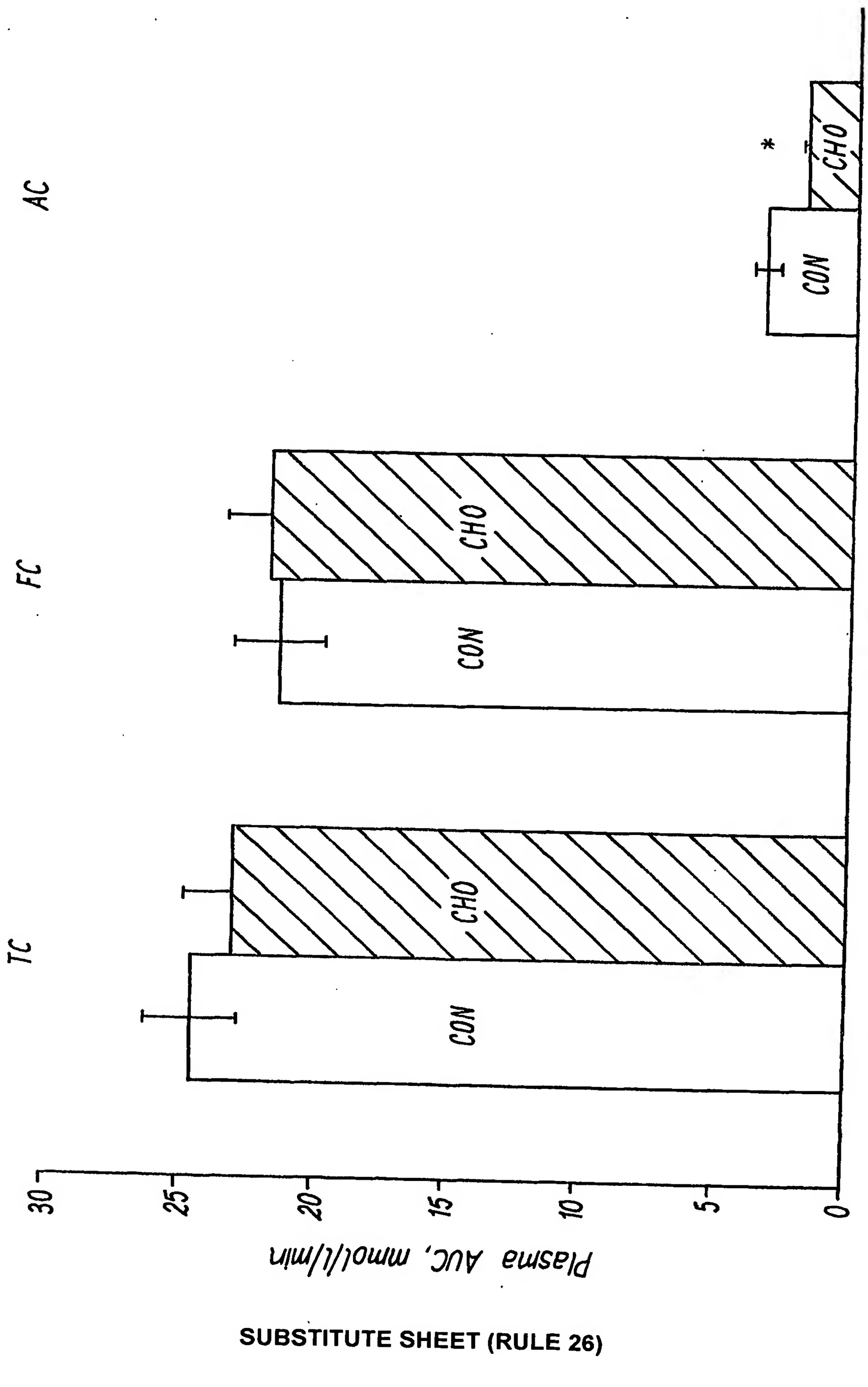

AC
FC
TC
CHO
CON
CHO
CON
CHO
CON
30
25
20
15
10
5
0
Plasma AUC, mmol/l/min

Fig 4

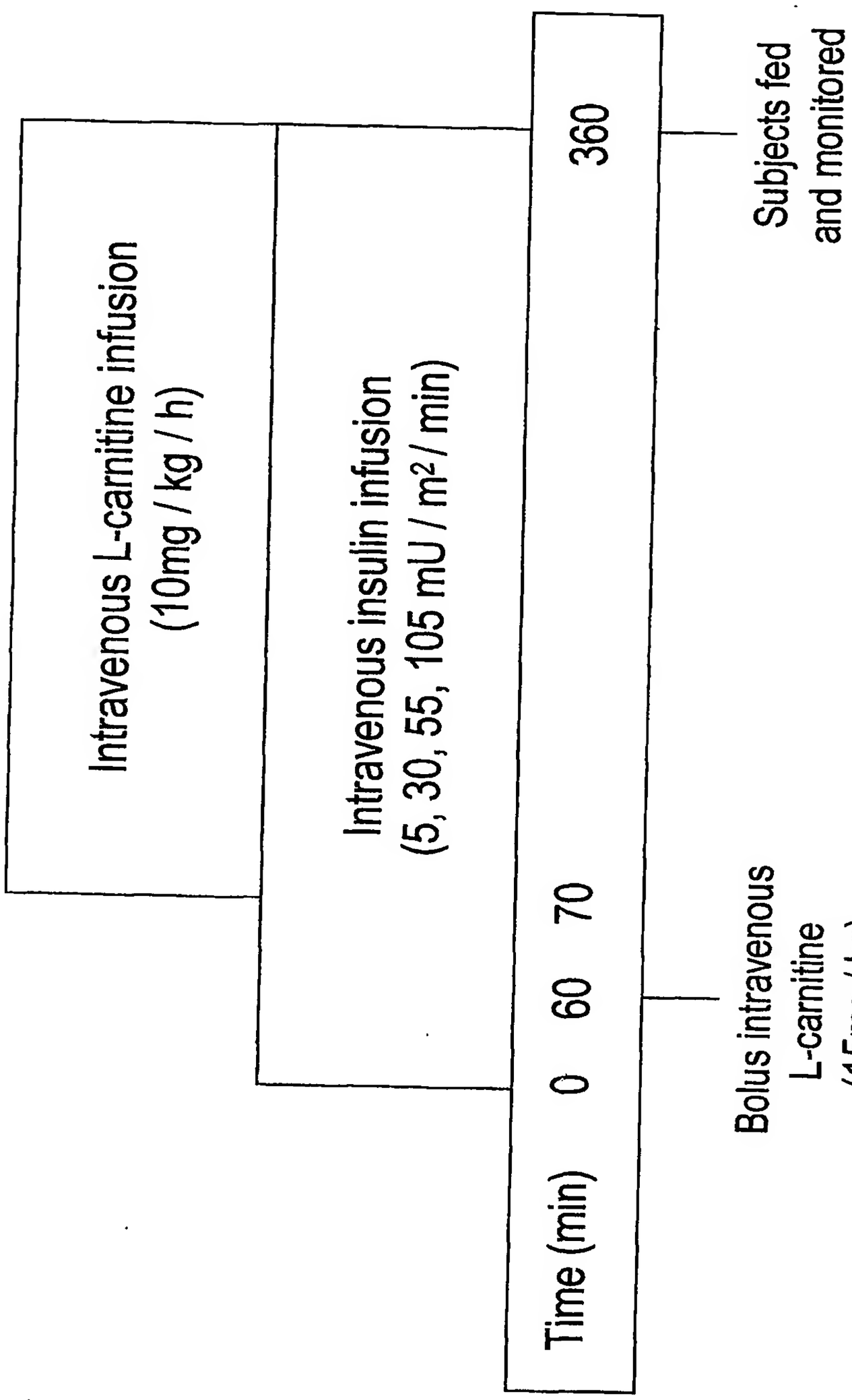
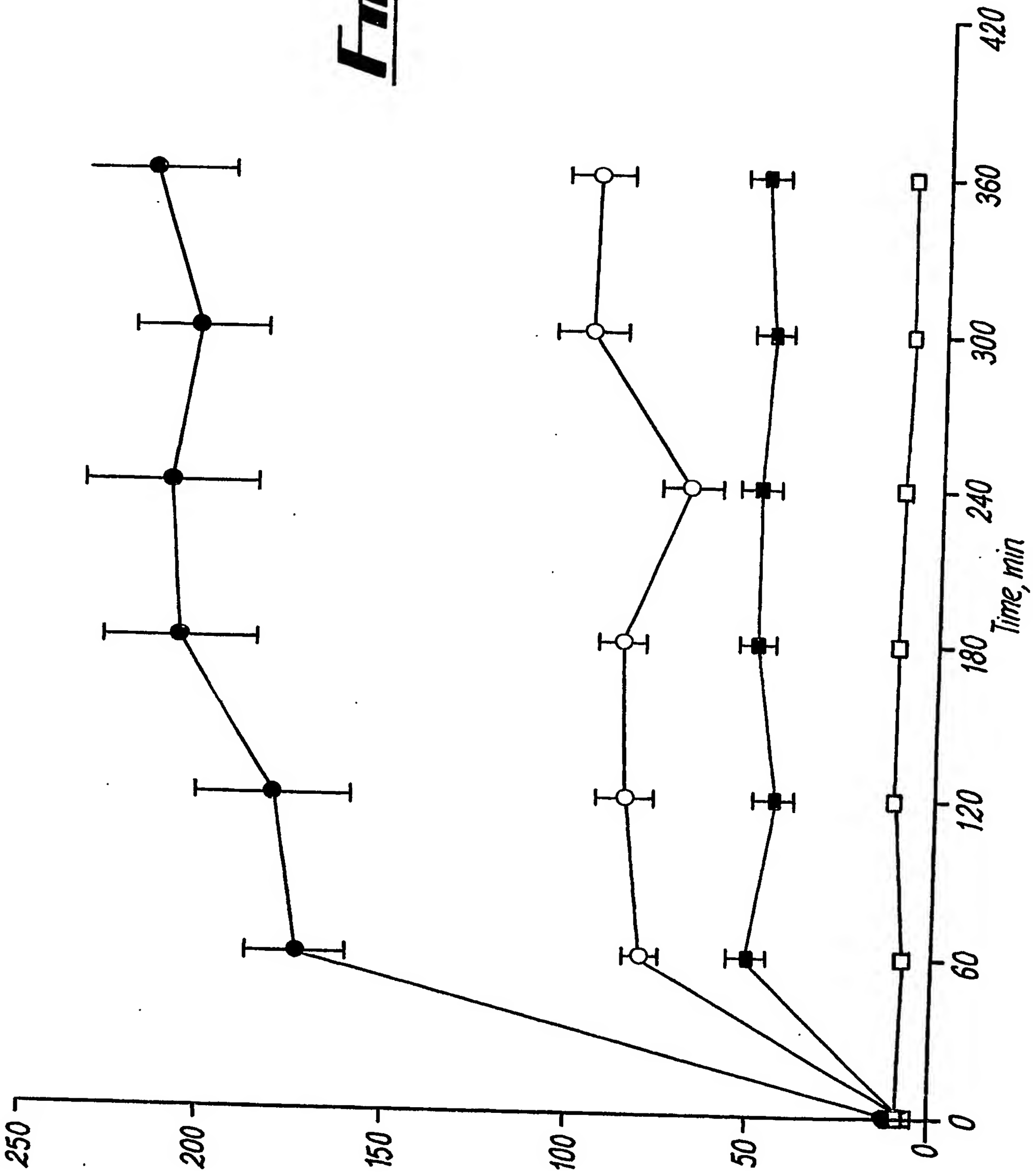

Intravenous L-carnitine infusion
(10mg / kg / h)
Intravenous insulin infusion
(5, 30, 55, 105 mU / m² / min)
0 60 70
Time (min)
Bolus intravenous
L-carnitine
(15mg / kg)
360
Subjects fed
and monitored

Fig 5

Fig 6



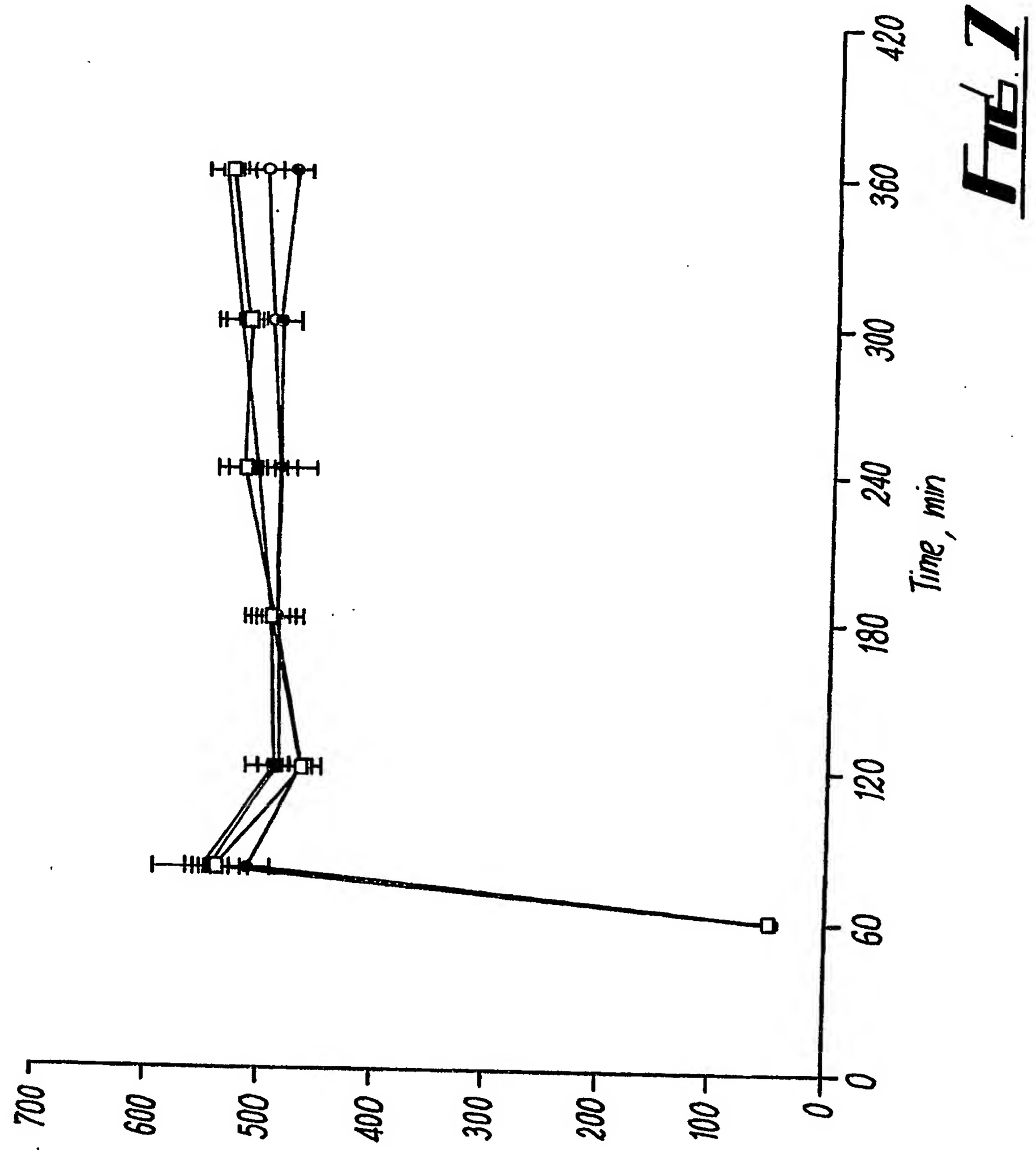
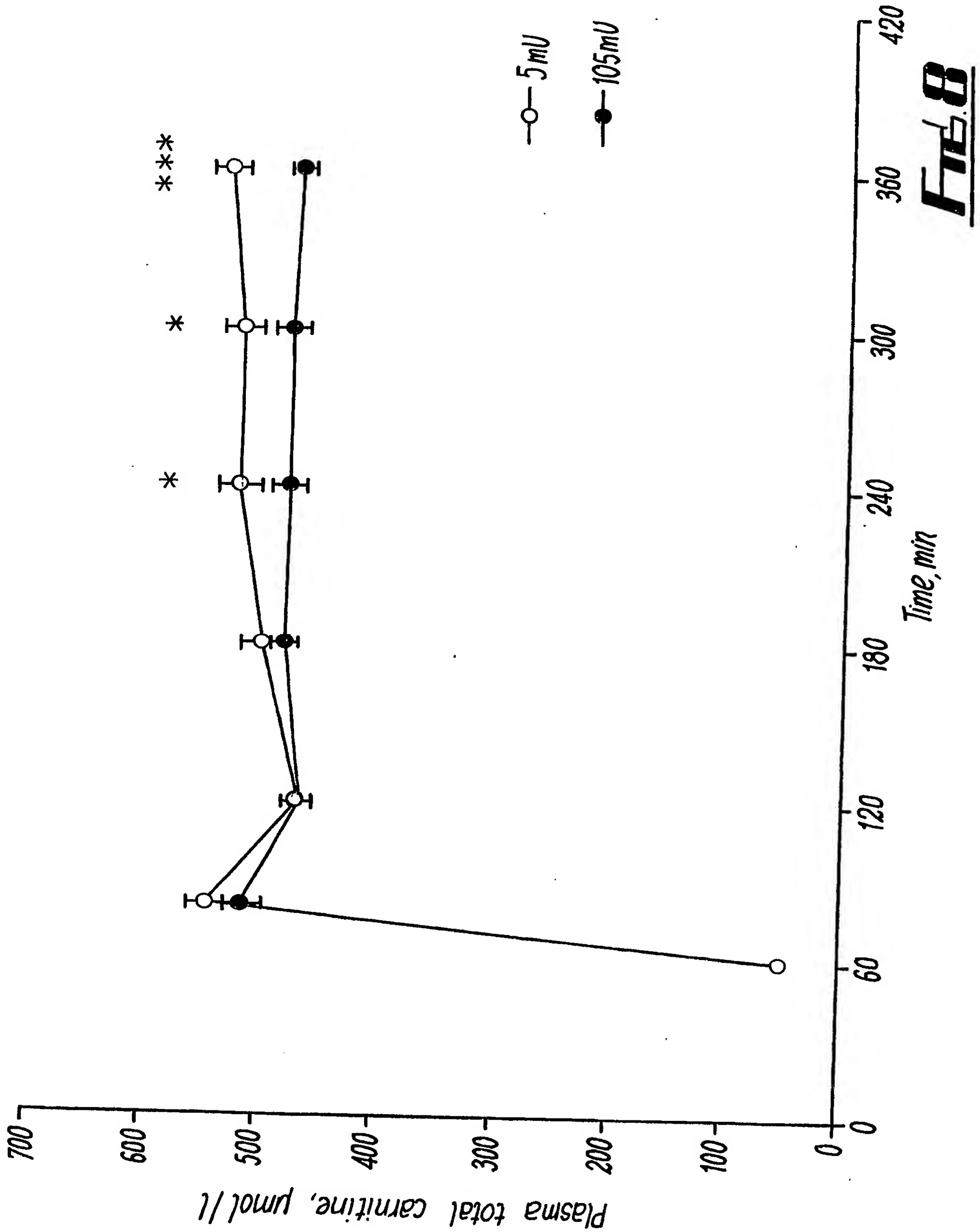

700
600
500
400
300
200
100
0
0
60
120
180
240
300
360
420
Time, min

FIG. 7


5mU
105mU
Fig.8
420
360
300
240
180
120
60
0
Time, min
700
600
500
400
300
200
100
0
Plasma total carnitine, μmol/l

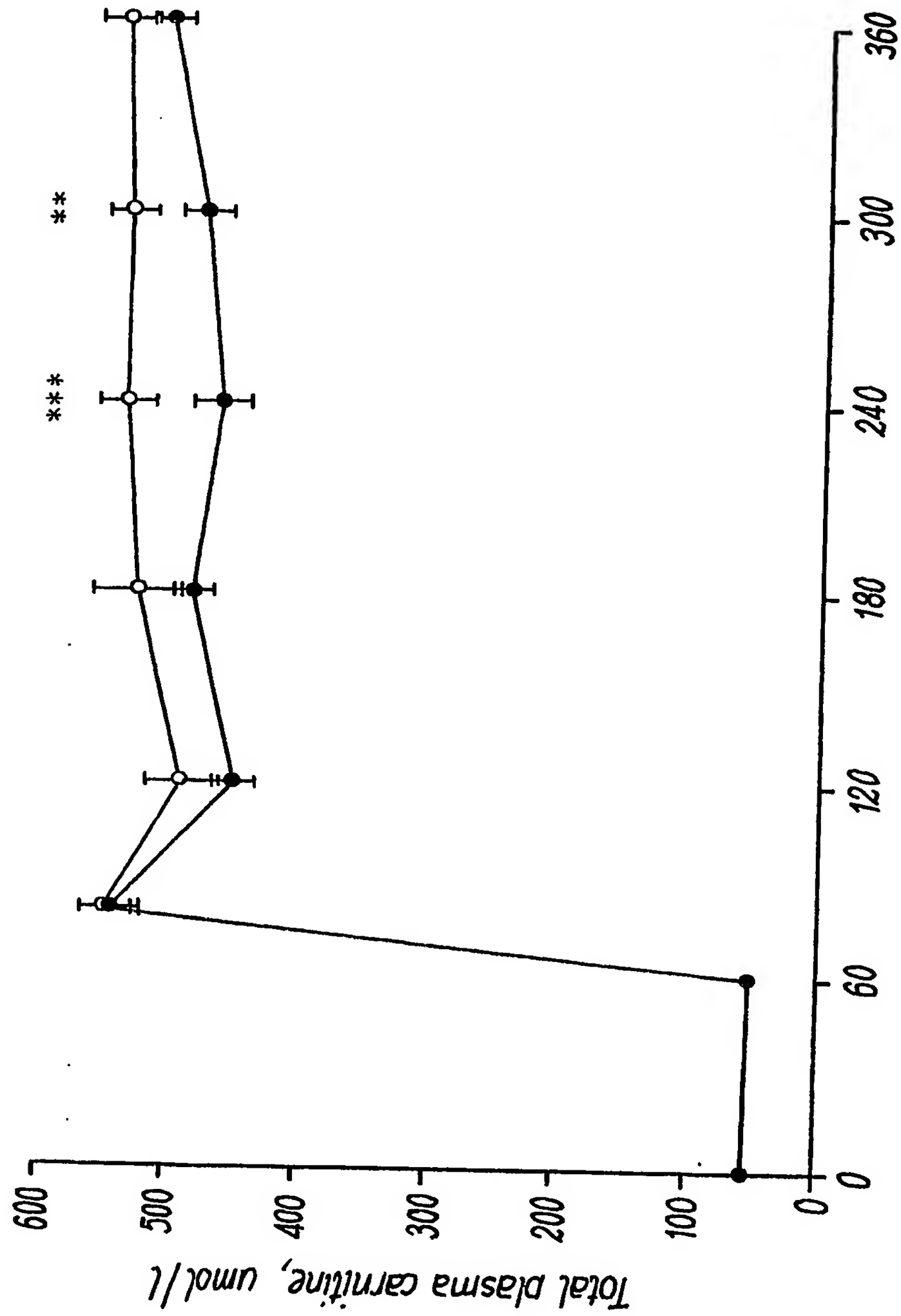

5mU
105mU
***
***
Total plasma carnitine, umol/l
600
500
400
300
200
100
0
0
60
120
180
240
300
360
Time, min

Fig. 9

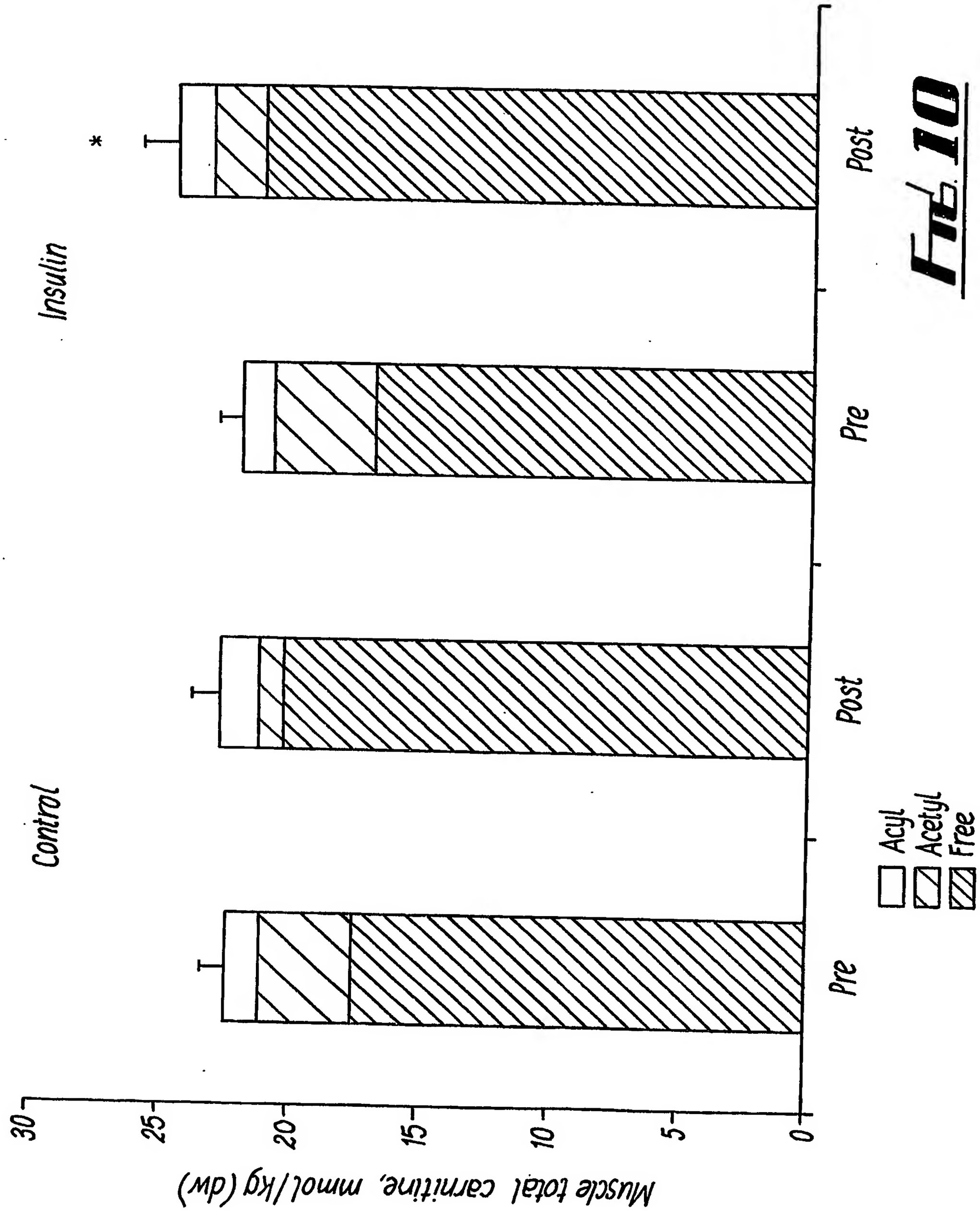

Insulin
Control
Pre
Post
Pre
Post
*
Acyl
Acetyl
Free
30
25
20
15
10
5
0
Muscle total carnitine, mmol/kg (dw)

Fig 10

INTERNATIONAL SEARCH REPORT

national Application No
Γ/GB2004/001256

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/205 A23L1/305

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, FSTA, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 680 945 A (OMEARA PTY LTD) 8 November 1995 (1995-11-08) claims 1,2,4,8,10; examples 1-7 page 2, line 39 - page 3, line 47 page 4, lines 36-51 ----- -/--	1-66

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2004	12/07/2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Tallgren, A

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

ıational Application No
. . :/GB2004/001256

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GROSS C J ET AL: "Effect of development and nutritional state on the uptake, metabolism and release of free and acetyl-L-carnitine by the rodent small intestine." BIOCHIMICA ET BIOPHYSICA ACTA. 3 NOV 1993, vol. 1170, no. 3, 3 November 1993 (1993-11-03), pages 265-274, XP008032228 ISSN: 0006-3002 page 265, paragraph 1 - page 266, paragraphs 1,6,7 tables 1-3 page 268, paragraph 1 - page 270, paragraph 3 page 272, paragraph 3 - page 273, paragraphs 2,4,5	1-22, 42-62
X	BOHLES H ET AL: "Improved N-retention during L-carnitine-supplemented total parenteral nutrition." JPEN. JOURNAL OF PARENTERAL AND ENTERAL NUTRITION. 1984 JAN-FEB, vol. 8, no. 1, January 1984 (1984-01), pages 9-13, XP008032205 ISSN: 0148-6071 page 9, paragraphs 1,2,5 page 11, paragraphs 3,6,8 - page 12, paragraph 2	1-22, 42-66
X	GREENWOOD R H ET AL: "Effects of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers." JOURNAL OF ANIMAL SCIENCE. JAN 2001, vol. 79, no. 1, January 2001 (2001-01), pages 254-260, XP002286196 ISSN: 0021-8812 page 254, paragraphs 1,4 page 256, paragraph 5 page 257, paragraphs 2,4,6 page 259, paragraph 5 - page 260, paragraphs 1,3 tables 1,2	1-22, 42-66

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INTERNATIONAL SEARCH REPORT

ational Application No
.../GB2004/001256

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LACOUNT D W ET AL: "Responses of dairy cows during early lactation to ruminal or abomasal administration of L-carnitine." August 1995 (1995-08), JOURNAL OF DAIRY SCIENCE. AUG 1995, VOL. 78, NR. 8, PAGE(S) 1824 - 1836 , XP002286406 ISSN: 0022-0302 page 1824, paragraph 1 page 1825, paragraph 3 table 1 page 1827, paragraph 3-5 page 1828, paragraph 3 - page 1829, paragraph 2 page 1835, paragraph 2	1-22, 42-66
X	LACOUNT D W ET AL: "Dose response of dairy cows to abomasal administration of four amounts of L-carnitine." April 1996 (1996-04), JOURNAL OF DAIRY SCIENCE. APR 1996, VOL. 79, NR. 4, PAGE(S) 591 - 602 , XP002286407 ISSN: 0022-0302 page 591, paragraph 3 page 593, paragraph 3 page 594, paragraph 3 page 596, paragraph 3 page 597, paragraphs 3,4 page 601, paragraph 2 tables 1,2,4,7	1-22, 42-66
X	LACOUNT D W ET AL: "Ruminal degradation and dose response of dairy cows to dietary L-carnitine." February 1996 (1996-02), JOURNAL OF DAIRY SCIENCE. FEB 1996, VOL. 79, NR. 2, PAGE(S) 260 - 269 , XP002286408 ISSN: 0022-0302 page 260, paragraphs 1,2,4 - page 261, paragraph 2 table 1 page 262, paragraph 2 page 263, paragraphs 4,5 page 267, paragraph 3	1-22, 42-66
X	WO 01/95915 A (SIGMA TAU HEALTHSCIENCE SPA ; POLA PIETRO (IT)) 20 December 2001 (2001-12-20) claims 1,3,9-14 page 1, paragraph 1 - page 2, paragraph 1 page 6, paragraph 3 - page 9, paragraph 1	1-41

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 42-66 (partially)

Rule 39.1(v) PCT - Presentation of information
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box II.2

Claims Nos.: 1-9, 23-28,42-44,47-49

Claims 1-9,23-28,42-44, 47-49 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the agents comprising carbohydrates, amino acids and proteins as disclosed in page 4 lines 1-2, page 5 lines 15-18, page 7 lines 19-22, claim 10.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/GB2004/001256

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 42-66 (partially)
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(v) PCT - Presentation of information
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [X] Claims Nos.: 1-9, 23-28,42-44,47-49
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
I/GB2004/001256

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